33. (Amended) The method of claim 32, wherein the human marrow stromal cells are incubated for at least about 10 days.

34. (Amended) The method of claim 32, wherein the colony-forming efficiency is compared with the colony-forming efficiency of another sample of human marrow stromal cells incubated in the same manner, wherein the expandability of the human marrow stromal cells of the other sample is known.

REMARKS

The present invention relates to methods for isolating and expanding human marrow stromal cells *in vitro*.

Claims 1-29 and 31-36 are under consideration.

Claims 1-19, 22 and 32-34 have been amended herein. Support for these amendments is found throughout the specification as filed and as more fully set forth below. Therefore, no new matter has been added by way of these amendments.

Preliminarily, Applicants respectfully call the Examiner's attention to the Revocation and Power of Attorney by Assignee which was filed in this application on April 5, 2001. A copy of the Revocation is enclosed herewith. This Revocation changed the address of correspondence to Kathryn Doyle at:

Morgan, Lewis & Bockius, L.L.P.
1701 Market Street
Philadelphia PA 19103.

Rejection of claims 1-29 and 31-36 pursuant to 35 U.S.C. § 112, second paragraph

Claims 1-29 and 31-36 stand rejected under 35 U.S.C. § 112, second paragraph, because in the view of the Examiner, they are indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

The Examiner asserts that claim 1 is indefinite in the recitation of "the isolated cells" in lines 1 and 6 because there is insufficient antecedent basis. Applicants have amended

claim 1 to clarify the antecedent basis by using the term "human marrow stromal cells" throughout, and to add reference to step numbers. The antecedent basis of "the isolated cells" is now clear.

The Examiner asserts that claims 3-11 are indefinite in the limitation "the cells" because the antecedent basis is unclear. Applicants have amended claims 3-11 to clarify the antecedent basis by using the term "human marrow stromal cells" throughout and to add reference to step numbers. Claims 3-9 now recite "in step (1) the initial density of the isolated human marrow stromal cells" which has clear antecedent basis in the "initial density of the isolated human marrow stromal cells" of claim 1. The phrase "after step (2) the human marrow stromal cells are harvested" of claims 10-11 has clear antecedent basis in the harvested human marrow stromal cells of claim 1, step (2).

The Examiner asserts that claim 12 is indefinite in the recitation of "harvested cells" in line 1 because there is insufficient antecedent basis. Claim 12 the "harvested cells" of claim 12, line 1 has been amended to recite "the proliferated human marrow stromal cells of step(2) are harvested." This amendment is supported by the specification (specification, page 22, line 2) and adds no new matter. The term "harvested cells" in the third line of step (3) in claim 12 finds antecedent basis in first line of step (3) of claim 12. Claim 12 has also been amended to recite "harvested human marrow stromal cells" to clarify antecedent basis.

The Examiner asserts that claim 12 is indefinite in the final recitation of "the cells" because there is insufficient antecedent basis. Applicants have amended claim 12 to recite "the human marrow stromal cells on the second growth surface proliferate." Applicants assert that there is now clear antecedent basis of the term "the cells."

The Examiner asserts that claim 13 is indefinite in the recitation "the cells" because there is insufficient antecedent basis. Claim 13 has been amended to recite "the harvested human marrow stromal cells are seeded on the second growth surface." There is now clear antecedent basis for the term "the cells" in claim 13.

In order to further clarify the antecedent basis of "the cells", the steps of the method have been numbered in claims 1, 12, and 16, and claims 2-11, 13-15, 17-19 and 22 have been amended to refer to the numbered steps in the claim limitations. Claims 1-19, 32-34 have been amended to recite "human marrow stromal cells throughout." These amendments merely clarify the antecedent basis of terms in the claims, and do not add new matter.

Claim 32 has been amended to recite "isolated human marrow stromal cells." Support for this amendment is found in the specification (specification, page 31, lines 11-12), and no new matter is added.

Applicants request that the rejection of claims 1-29 and 31-36 under § 112, second paragraph, be reconsidered and withdrawn.

Rejection of claims 1-21 pursuant to 35 U.S.C. § 102(b)

Claims 1-21 stand rejected under 35 U.S.C. § 102(b), because in the view of the Examiner, they are anticipated by Bruder et al. (1997, J. of Cell. Biochem. 64:278-294). Specifically, the Examiner asserts that Bruder teaches that when bone marrow cells are plated onto tissue culture dishes at a density of 10^7 cells per 60 cm^2, adherent mesenchymal cells represent approximately 1 cell per 10^5 nucleated cells. Applicants respectfully traverse this rejection.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

Bruder does not teach every element of claims 1-21. Step (1) of claim 1 recites providing "isolated human marrow stromal cells" to a growth surface such that the initial density is less than about 50 cells per square centimeter of growth surface. The specification defines "isolated MSCs" as "MSCs that have been separated from at least certain other bone marrow cells with which they normally occur (e.g. in bone marrow)" (specification, page 18, lines 7-9). The specification further discloses that one method of preparing "isolated MSCs" is by their adherence to plastic (specification, page 28, lines 20-21; page 29, lines 22-27; and other places). Bruder describes a procedure for the isolation of MSC from marrow aspiratants (Bruder, page 280, column 1, lines 1-3) by plating at 10^7 cells per 60 cm^2 and harvesting the adherent cells (Bruder, page 280, column 1, first paragraph). Bruder does not teach a method where isolated MSC cells are plated at initial densities less than about 50 cells per cm^2. Bruder does not teach a method where the MSCs are harvested from the first growth surface and provided to a second growth surface such that the density is not more than about 50 cells per cm^2. Bruder does not teach a method where the MSCs are harvested from the second growth surface and provided to a third growth surface such that the density is not more than about 50 cells per cm^2.

For the reasons set forth above, Applicants respectfully request that the rejection of claims 1-21 under 35 U.S.C. § 102(b) be reconsidered and withdrawn.

Rejection of claims 1-21 pursuant to 35 U.S.C. § 102(b)

Claims 1-21 stand rejected under 35 U.S.C. § 102(b), because in the view of the Examiner, they are anticipated by Kuznetsov et al. (1997, J. of Bone and Mineral Research, 12:1335-1347). Specifically, the Examiner asserts that Kuznetsov teaches single colony derived strains of human marrow stromal fibroblast cells. Applicants respectfully traverse this rejection.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

Kuznetsov does not teach every element of claims 1-21. Step (1) of claim 1 recites providing "isolated human marrow stromal cells" to a growth surface such that the initial density is less than about 50 cells per square centimeter of growth surface. The specification defines "isolated MSCs" as "MSCs that have been separated from at least certain other bone marrow cells with which they normally occur (e.g. in bone marrow)" (specification, page 18, lines 7-9). The specification further discloses that one method of preparing "isolated MSCs" is by their adherence to plastic (specification, page 28, lines 20-21; page 29, lines 22-27; and other places). Kuznetsov teaches a method where marrow cells are plated at densities of 0.14 – 14.0 x 10^3 cells/cm^2 or (Kuznetsov, page 1337, column 1, lines 1-2 of second paragraph). Kuznetsov does not teach a method where isolated MSC cells are plated at initial densities less than about 50 cells per cm^2. Kuznetsov does not teach a method where the MSCs are harvested from the first growth surface and provided to a second growth surface such that the density is not more than about 50 cells per cm^2. Kuznetsov does not teach a method where the MSCs are harvested from the second growth surface and provided to a third growth surface such that the density is not more than about 50 cells per cm^2.

In view of the fact that each and every element of the claim is not found in Kuznetsov, Applicants respectfully request that the rejection of claims 1-21 under 35 U.S.C. § 102(b) be reconsidered and withdrawn.

Rejection of claims 1-21 pursuant to 35 U.S.C. § 102(a)

Claims 1-21 stand rejected under 35 U.S.C. § 102(a), because in the view of the Examiner, they are anticipated by DiGirolamo et al., (1999, British Journal of Haematology, 107:275-281). Applicants respectfully traverse this rejection. DiGirolamo is not a prior art reference.

A person shall be entitled to a patent unless (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent. 35 U.S.C. 102(a). The filing of a patent application serves as conception and constructive reduction to practice of the subject matter described in the application. Thus the inventor need not provide evidence of either conception or actual reduction to practice when relying on the content of the patent application. *Hyatt v. Boone*, 146 F.3d 1348, 1352, 47 USPQ2d 1128, 1130 (Fed. Cir. 1998). A publication disseminated by mail is not prior art until it is received by at least one member of the public. Thus, a magazine or technical journal is effective as of its date of publication (date when first person receives it) not the date it was mailed or sent to the publisher. *In re Schlittler*, 234 F.2d 882, 110 USPQ 304 (CCPA 1956).

The DiGirolamo reference is found in Issue 2 of Volume 107 of the British Journal of Haematology, dated November 1999. This issue was received by the public on or about November 17, 1999, as evidenced by the stamped cover of Issue 2 received by Scott Library at Thomas Jefferson University, enclosed herewith. The present application claims the benefit of provisional application 60/162,474, filed October 29, 1999. Therefore, for the invention to be anticipated by a publication under 35 U.S.C. § 102(a), the publication must be available to the public before October 29, 1999, the effective filing date of the present application. The DiGirolamo publication was not available to the public until several weeks after the filing date of the present application, and therefore, DiGirolamo does not anticipate the present invention.

For the reasons set forth above, Applicants request that the rejection of claims 1-21 under 35 U.S.C. § 102(a) be reconsidered and withdrawn.

Rejection of claims 1 and 22-23 pursuant to 35 U.S.C. § 103(a)

Claims 1 and 22-23 stand rejected under 35 U.S.C. § 103(a), because in the view of the Examiner, they are unpatentable over Kuznetsov et al. (1997, J. of Bone and Mineral Research, 12:1335-1347) in view of Azizi et al. (1998, Proc. Natl. Acad. Sci. 95:3908-3913). Specifically, the Examiner asserts that Kuznetsov teaches single colony derived strains of human marrow stromal fibroblast cells, multiple passaging and cell culture comprising fetal bovine serum. The Examiner asserts that Azizi teaches growing human marrow stromal cells with the addition of PDGF-AA to the culture. Applicants respectfully traverse this rejection because the claimed invention is not obvious in light of the teachings of the references.

The three-prong test which must be met for a reference or a combination of references to establish a *prima facie* case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

None of these criteria have been met here.

There is no suggestion or motivation to modify or combine the teachings of Kuznetsov and Azizi in the references themselves or in the knowledge generally available to one of ordinary skill in the art. Kuznetsov teaches using bone marrow stromal fibroblasts to generate bone tissue *in vivo* (Kuznetsov, page 113, column 1, second paragraph). Azizi teaches using bone marrow stromal cells to form brain tissue *in vivo* (Azizi, abstract). One of skill in the art would not be motivated to combine these references because to goals of the references are so different. Furthermore, the teachings of a reference directed to bone generation would be considered unlikely by one of skill in the art to have useful information for the formation of brain tissue. Likewise, a reference directed to brain tissue formation would be considered unlikely by one of skill in the art to have useful information for the formation of bone tissue. One of skill in the art would therefore not be motivated to combine Kuznetsov and Azizi.

There is no reasonable expectation of success in combining the teachings of Kuznetsov and Azizi. Brain tissue and bone tissue are composed of different cells. One of skill in the art would not reasonably expect success with using techniques developed for the generation of brain tissue *in vivo* when used for the generation of bone tissue *in vivo*. Likewise, one of skill in the art would not expect success with using techniques developed for the generation of bone tissue *in vivo* when used for the generation of brain tissue *in vivo*. Therefore, should one be motivated to combine Kuznetsov and Azizi, they would not have a reasonable expectation of success in the combination.

Finally, Kuznetsov and Azizi, taken individually or together, do not teach or suggest all claim limitations. Kuznetsov and Azizi, taken individually or together, do not teach a method where isolated MSC cells are plated at initial densities of less than about 50 cells per cm^2. The specification defines "isolated MSCs" as "MSCs that have been separated from at least certain other bone marrow cells with which they normally occur (e.g. in bone marrow)" (specification, page 18, lines 7-9). The specification further discloses that one method of preparing "isolated MSCs" is by their adherence to plastic (specification, page 28, lines 20-21; page 29, lines 22-27; and other places). Kuznetsov teaches a method where marrow cells are plated at densities of $0.14 - 14.0 \times 10^3$ cells/cm^2 or (Kuznetsov, page 1337, column 1, lines 1-2 of second paragraph). Kuznetsov does not teach a method where isolated MSC cells are plated at initial densities less than about 50 cells per cm^2. Azizi teaches the plating of nucleated cells from aspirates from the iliac crest at 3×10^6 cells per cm^2 (Azizi, page 3904, column 2, second paragraph). If one of skill in the art were to combine Kuznetsov and Azizi, they would not make the claimed invention because not all of the claim limitations are taught in these references.

Applicants respectfully request that the rejection of claims 1 and 22-23 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

Rejection of claims 1, 24-29 and 31-36 pursuant to 35 U.S.C. § 103(a)

Claims 1, 24-29 and 31-36 stand rejected under 35 U.S.C. § 103(a), because in the view of the Examiner, they are unpatentable over Kuznetsov et al. and Azizi et al. in view of Greenberger et al. (U.S. Patent No. 5,766,950) and Prockop et al. (1997, Science 276:71-74). The Examiner asserts that Kuznetsov teaches single colony derived strains of human marrow stromal fibroblast cells, multiple passaging and cell culture comprising fetal bovine serum, Azizi

teaches growing human marrow stromal cells with the addition of PDGF-AA to the culture, Greenberger teaches a method for selection and expansion of stromal cells wherein the cells are grown in a vessel pre-coated with fibroblast growth factor and the cell culture is maintained in the presence of conditioned medium, and Prockop teaches the general state of the art in the field of marrow stromal cell culture. Applicants respectfully traverse this rejection because one of skill in the art would not find the invention obviousness in light of the teachings of the references. None of the criteria to establish a *prima facie* case of obviousness has been established. .

There is no suggestion or motivation to modify or combine the teachings of Kuznetsov, Azizi, Greenberger and Prockop in the references themselves or in the knowledge generally available to one of ordinary skill in the art. Kuznetsov teaches using bone marrow stromal fibroblasts to generate bone tissue *in vivo* (Kuznetsov, page 113, column 1, second paragraph). Azizi teaches using bone marrow stromal cells to form brain tissue *in vivo* (Azizi, abstract). Greenberger teaches the culturing of bone marrow stromal cells (Greenberger, abstract). Prockop teaches a review of the literature concerning marrow stromal cells as stem cells for nonhematopoietic tissues (Prockop, abstract). There is no motivation to combine Greenberger with Kuznetsov, Azizi or Prockop because Greenberger teaches that bone marrow transplantation is useful for treatment of diseases that involve hematopoietic cells (Greenberger, column 1, lines 50-52), while the bone cells of Kuznetsov and the brain cells of Azizi are nonhematopoietic cells, and Prockop reviews the literature with regard to nonhematopoietic tissues.

There is no reasonable expectation of success in combining the teachings of Kuznetsov, Azizi, Greenberger and Prockop. Because Greenberger teaches that bone marrow stromal cells are useful for generating hematopoietic cells, one of skill in the art would not expect success in combining it with Kuznetsov, Azizi and Prockop to make nonhematopoietic tissues. Because Prockop does not teach the details of techniques, one of skill in the art would not expect success in combining this reference with the experimental techniques of Kuznetsov, Azizi and Greenberger. Because bone tissue and brain tissue are very different, one of skill in the art would not expect success in using techniques developed for the generation of brain tissue *in vivo* in Kuznetsov when used for the generation of bone tissue *in vivo* in Azizi, nor the techniques developed for the generation of bone tissue *in vivo* in Azizi when used for the

generation of brain tissue *in vivo* in Kuznetsov. Therefore, should one be motivated to combine Kuznetsov, Azizi, Greenberger and Prockop, they would have no reasonable expectation of success in the combination.

Kuznetsov, Azizi, Greenberger and Prockop, taken individually or together, do not teach or suggest every limitation of claims 1, 24-29, and 32-36. Kuznetsov, Azizi, Greenberger and Prockop, taken individually or together, do not teach a method where isolated MSC cells are plated at initial densities less than about 50 cells per cm^2. The specification defines "isolated MSCs" as "MSCs that have been separated from at least certain other bone marrow cells with which they normally occur (e.g. in bone marrow)" (specification, page 18, lines 7-9). The specification further discloses that one method of preparing "isolated MSCs" is by their adherence to plastic (specification, page 28, lines 20-21; page 29, lines 22-27; and other places). Kuznetsov teaches a method where marrow cells are plated at densities of 0.14 – 14.0 x 10^3 cells/cm^2 or (Kuznetsov, page 1337, column 1, lines 1-2 of second paragraph). Kuznetsov does not teach a method where isolated MSC cells are plated at initial densities less than about 50 cells per cm^2. Azizi teaches the plating of nucleated cells from aspirates from the iliac crest at 3 x 10^6 cells per cm^2 (Azizi, page 3904, column 2, second paragraph). Greenberger teaches that 1 x 10^8 cells are added to T150 flasks (Greenberger, column 6, lines 12-15), which have 150 cm^2 surface area (Greenberger, column 6, line 1), so that 6.7 x 10^5 cells are provided per cm^2. Greenberger teaches bone marrow cells, not isolated stromal cells, are provided to the growth surface (Greenberger, column 6, line 12). If one of skill in the art were to combine Kuznetsov, Azizi, Greenberger and Prockop, they would not make the claimed invention because not all of the limitations of claims 1, 24-29, and 32-36 are taught in these references.

Kuznetsov, Azizi, Greenberger and Prockop, taken individually or together, do not teach every limitation of claims 24-29. Kuznetsov, Azizi, Greenberger and Prockop, taken individually or together, do not teach a method for enhancing the *in vitro* production of isolated marrow stromal cells by supplementing the growth medium with a factor present in a conditioned medium, wherein the conditioned medium is obtained from a culture of producer human marrow stromal cells which are grown on a second surface at an initial density of at least about 0.5 cells per square centimeter. The Examiner alleges that Greenberger teaches a method to grow MSCs where the cell culture is maintained in the presence of conditioned medium. Greenberger teaches that the "conditioned medium" is obtained from the bone marrow cell

culture, diluted 1:1 with fresh medium, then "returned to the original flask" (Greenberger, column 6, lines 20-29). Therefore, the conditioned medium of Greenberger is not derived from a "second surface." The growth surface in Greenberger is provided with 6.7×10^5 cells per cm^2, so that the cells are present at 0.5 cells per square centimeter on the growth surface providing the conditioned medium. If one of skill in the art were to combine Kuznetsov, Azizi, Greenberger and Prockop, they would not make the claimed invention because not all of the limitations of claims 24-29 are taught in these references.

Kuznetsov, Azizi, Greenberger and Prockop, taken individually or together, do not teach every limitation of claims 28, 29 and 31-36. Kuznetsov, Azizi, Greenberger and Prockop, taken individually or together, do not teach a method for enhancing the *in vitro* production of isolated marrow stromal cells by supplementing the growth medium with a fraction of the conditioned medium containing size fractionated molecules having a molecular weight of about 30,000 or about 10,000. Kuznetsov, Azizi, Greenberger and Prockop, taken individually or together, do not teach a method for inducing proliferation of human marrow stromal cells comprising isolating an individual colony of marrow stromal cells and incubating those cells in a container at an initial density of less than about 50 cells per cm^2. Kuznetsov, Azizi, Greenberger and Prockop, taken individually or together, do not teach a method for accessing the expandability of human marrow stromal cells by accessing the colony forming efficiency of the cells. If one of skill in the art were to combine Kuznetsov, Azizi, Greenberger and Prockop, they would not make the claimed invention because not all of the limitations of claims 28, 29 and 31-36 are taught in these references.

In light of the lack of teaching of various elements of the invention by the references, one of skill in the art would not find the claimed invention to be obvious in light of the teachings of the references. Applicants respectfully request that the rejection of claims 1, 24-29 and 31-36 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been overcome or is now inapplicable, and that each of currently pending claims 1-29 and 31-36 is in condition for allowance. Reconsideration and allowance of claims 1-29 and 31-36 are respectfully requested at the earliest possible date.

Respectfully submitted,

PROCKOP *ET AL.*

4 Dec 2002
(Date)

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Enclosures (Copy of Revocation and Power of Attorney; cover and index pages form British Journal of Haemaology Vol. 107 No. 2, petition for three-month extension of time and fee therefor; "marked-up" copy of the claims)

Marked-Up Copy of Claims

1. (Amended) A method of inducing proliferation of isolated human marrow stromal cells in vitro, the method comprising

(1) providing the isolated human marrow stromal cells and a growth medium to a growth surface such that the initial density of the isolated human marrow stromal cells is less than about 50 cells per square centimeter of growth surface, and

(2) incubating the growth surface of step (1) under growth-promoting conditions, whereby the human marrow stromal cells proliferate.

2. (Amended) The method of claim 1, wherein in step (1) the initial density of the isolated human marrow stromal cells is less than about 25 cells per square centimeter of growth surface.

3. (Amended) The method of claim 1, wherein in step (1) the initial density of the isolated human marrow stromal cells is less than about 12 cells per square centimeter of growth surface.

4. (Amended) The method of claim 1, wherein in step (1) the initial density of the isolated human marrow stromal cells is less than about 10 cells per square centimeter of growth surface.

5. (Amended) The method of claim 1, wherein in step (1) the initial density of the isolated human marrow stromal cells is less than about 6 cells per square centimeter of growth surface.

6. (Amended) The method of claim 1, wherein in step (1) the initial density of the isolated human marrow stromal cells is less than about 3 cells per square centimeter of growth surface.

7. (Amended) The method of claim 1, wherein in step (1) the initial density of the isolated human marrow stromal cells if less than about 1.5 cells per square centimeter of growth surface.

8. (Amended) The method of claim 1, wherein in step (1) the initial density of the isolated human marrow stromal cells is less than about 1.0 cells per square of growth surface.

9. (Amended) The method of claim 1, wherein in step (1) the initial density of the isolated human marrow stromal cells is at least about 0.5 cells per square centimeter of growth surface.

10. (Amended) The method of claim 1, wherein after step (2) the human marrow stromal cells are harvested from the growth surface following not more than about 14 days of incubation.

11. (Amended) The method of claim 1, wherein after step (2) the human marrow stromal cells are harvested from the growth surface following not more than about 10 days of incubation.

12. (Amended) The method of claim 1, further wherein

(3) the proliferated human marrow stromal cells of step (2) are harvested [cells] and [a growth medium] are provided to a second growth surface along with a growth medium such that the initial density of the harvested cells is less than about 50 cells per square centimeter of second growth surface; [and]

(4) the second growth surface is incubated under growth-promoting conditions, whereby the human marrow stromal cells on the second growth surface proliferate; and

(5) the human marrow stromal cells on the second growth surface are harvested.

13. (Amended) The method of claim 12, wherein in step (3) the harvested human marrow stromal cells are seeded on the second growth surface at an initial density of about 3 cells per square centimeter.

14. (Amended) The method of claim 12, wherein in step (5) the human marrow stromal cells are harvested from the second growth surface following not more than about 14 days of incubation.

15. (Amended) The method of claim 12, wherein in step (5) the human marrow stromal cells are harvested from the second growth surface following not more than about 10 days incubation.

16. (Amended) The method of claim 12, further wherein

(6) cells harvested from the second growth surface in step (5) and a growth medium are provided to a third growth surface such that the initial density of the human marrow stromal cells harvested from the second growth surface is less than about 50 cells per square centimeter of the third growth surface and

(7) the third growth surface is incubated under growth-promoting conditions, whereby the human marrow stromal cells on the third growth surface proliferate; and

(8) the human marrow stromal cells on the third growth surface are harvested.

17. (Amended) The method of claim 16, wherein in step (8) the human marrow stromal cells are harvested from the third growth surface following not more than about 14 days of incubation.

18. (Amended) The method of claim 16, wherein in step (8) the human marrow stromal cells are harvested following not more than about 10 days of incubation.

19. (Amended) The method of claim 16, wherein in step (6) the human marrow stromal cells are seeded on the third growth surface at an initial density of about 3 cells per square centimeter.

22. (Amended) The method of claim 1, further wherein in step (1) a growth factor is added to the growth medium.

32. (Amended) [The] A method of assessing the expandability of isolated human marrow stromal cells *in vitro*, the method comprising incubating [the] isolated human marrow stromal cells on a surface in the presence of a growth medium at an initial density of less than about 50 cells per square centimeter of surface and assessing the colony-forming efficiency of the human marrow stromal cells, whereby the expandability of the human marrow stromal cells is approximately proportional to the colony-forming efficiency of the human marrow stromal cells.

33. (Amended) The method of claim 32, wherein the human marrow stromal cells are incubated for at least about 10 days.

34. (Amended) The method of claim 32, wherein the colony-forming efficiency is compared with the colony-forming efficiency of another sample of human marrow stromal cells incubated in the same manner, wherein the expandability of the human marrow stromal cells of the other sample is known.

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